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NATURE OF INHERITED RESISTANCE TO VIRUSES AFFECTING THE NERVOUS SYSTEM

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Introduction.—The vast majority of viruses which attack the nervous system of human beings and animals produce recognizable disease and death in only a small proportion of infected individuals. A variety of factors pertaining to the virus, the host, or both, may influence the outcome of infection. The studies to be presented here were designed to elucidate the intricate biological mechanisms which form the basis of this important phenomenon.

Previous studies by other investigators have established that plants and animals may possess an inherited resistance to various infectious and noxious agents, including viruses. The studies of Lynch and Hughes[1] with the virus of yellow fever and those of Webster[2] with the viruses of louping ill and St. Louis encephalitis provided the first experimental evidence that the genetic constitution of the host can determine the outcome of mammalian viral infections. A most important contribution to this subject was Webster's demonstration that the inherent resistance or susceptibility of mice to the virus of St. Louis encephalitis was correlated with the level of viral multiplication in the brain, not only in the intact animal[3] but also in simple cultures containing the minced brain tissue.[4] Earlier attempts to establish the manner in which such resistance is inherited by animals, and to segregate the factors which are genetically affected, were complicated by the fact that no uniformly resistant animals were available.

Experimental Results.—In 1944, the author accidentally discovered that the albino mice which had been bred for over 25 years at the Rockefeller Institute at Princeton, N. J., were 100 per cent resistant to the 17 D strain of yellow fever virus that is widely used for human vaccination. Swiss mice, intracerebrally inoculated with this virus, invariably die after exhibiting

paralysis and other signs of involvement of the nervous system. In the Princeton Rockefeller Institute mice (henceforth called PRI), intracerebral inoculation of the 17 D virus, in the largest as well as the smallest doses, results in multiplication at a level only $^1/_{10,000}$ to $^1/_{100,000}$ of that achieved in Swiss mice, and in the development of specific antibodies—but all mice survive without showing any clinical signs of disease. The possibility was considered that a latent virus in the PRI mice might interfere with the multiplication of the yellow fever virus, but none was found in tests with brain, viscera, intestines and feces. This circumstance presented an unusual opportunity for genetic analysis by the classical methods of

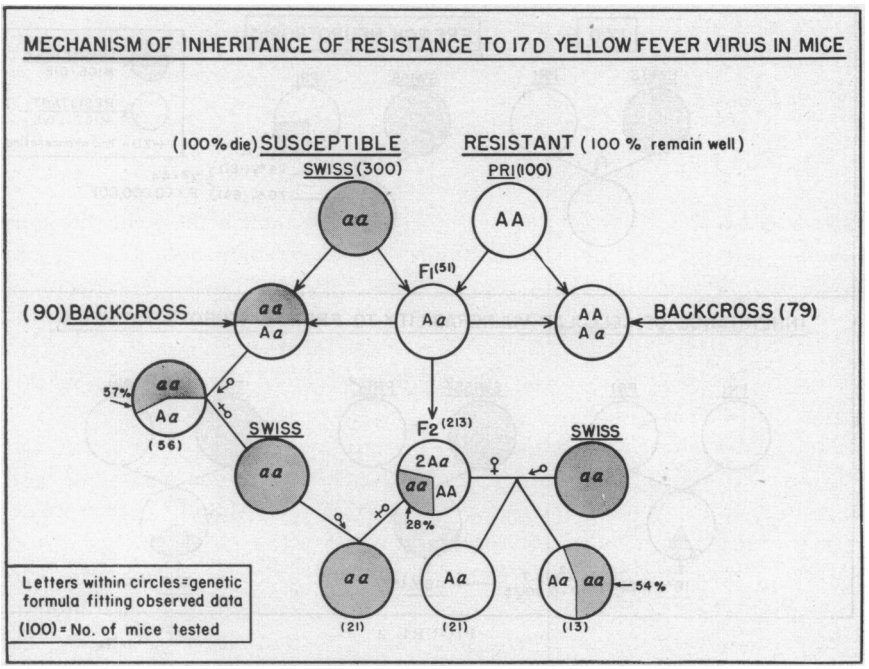

FIGURE 1

interbreeding. Such studies were begun in 1950, and this communication is a progress report of the results obtained thus far.

Figure 1 presents a diagrammatic summary of the tests with the 17 D virus. The sex of the parents and progeny is not indicated because no evidence of sex-linkage was found. Since all F_1 progeny survived without signs of disease, it is clear that resistance to this virus is inherited as a dominant. The approximate 3:1 ratio of resistants to susceptibles in the F_2 progeny, the exact 1:1 ratio in the backcross of F_1 to susceptible Swiss mice, and the 100 per cent resistance in the backcross of F_1 to resistant PRI mice, are all in accord with the Mendelian laws for a single pair of

autosomal genes. However, in 1934, Sewall Wright[5] demonstrated that such ratios are not critical criteria for the operation of a single pair of genes and that the critical experiment must test the genetic nature of the backcross and F_2 progeny by breeding them with the recessive stock. This was done, as shown in figure 1, and the results obtained were again compatible with a single factor hypothesis. It is realized, however, that it is difficult to rule out other possibilities involving multiple genes, which do not assort

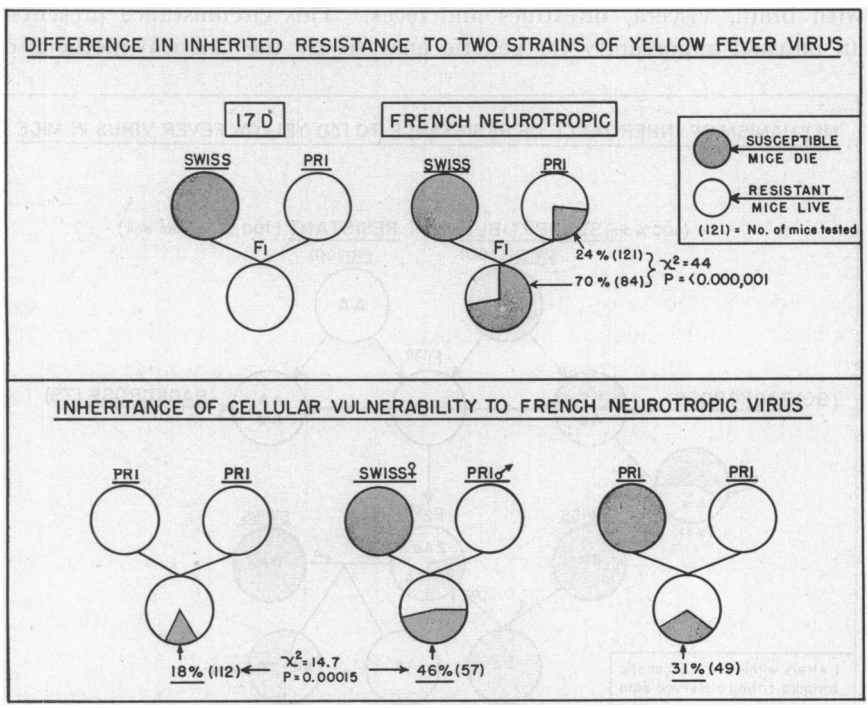

FIGURE 2

This figure shows the mortality resulting from tests on parental and F_1 groups of mice. The genetic aspects are discussed in the text.

at random in the gametes but are linked with only a limited amount of crossing over. Thus, if resistance to 17 D were dependent on two pairs of dominant, linked genes, with 10 per cent crossing over, the backcross and F_2 progeny and the progeny resulting from crossing these with the recessive stock would be modified so slightly from the single factor expectancy that only exceptionally large numbers of animals might yield results of sufficient statistical significance to permit a decision. However, whatever the exact number of genes may be, it is clear that the resistance of PRI mice to the 17 D virus depends on a factor, which depresses viral multiplication, and

is inherited as an "all or none" character, because the progeny which segregated out of the various crossings were phenotypically like the parent resistant or susceptible stock, i.e., the resistant mice showed no signs of disease and the susceptible ones exhibited the same incubation period and high level of viral multiplication as the Swiss mice.

All this held good as long as the tests were restricted to mature animals. When newborn, 1-day or 2-day old PRI mice were inoculated with 17 D virus, all mice died after an incubation period of 10 days or longer. PRI mice inoculated at 3 to 5 days of age behaved irregularly, and beyond 5 days of age, the suckling mice behaved like the mature animals. Tests for viral multiplication in the succumbing suckling PRI mice showed that the peak levels were as low as in adult PRI mice which remained well. It was apparent, therefore, that at low levels of viral multiplication the tissues of the newborn mice were more vulnerable than those of older mice.

The concept of cellular vulnerability again came under consideration when it was found that the "French neurotropic" strain of yellow fever virus killed a certain proportion of adult PRI mice. This strain of virus has had over 250 serial passages in the brains of Swiss mice, and although its level of multiplication is not significantly higher than that of the 17 D strain, it kills mice more rapidly. The results summarized in figure 2 show that 24 per cent of adult PRI mice died after intracerebral inoculation of the "French neurotropic" virus. The virus multiplied at the same low level in the PRI mice that died as in those that remained well, and both of these levels were about the same as the peak levels of the 17 D strain. There was no evidence that the virus particles which grew out in the brains of the dying mice were different from those in the brains of mice which remained well, since passage to new PRI mice yielded a mortality of only 20 per cent. It appeared, therefore, that some of the PRI mice were more vulnerable than others at similar low levels of viral multiplication, and the question arose as to whether cellular vulnerability might also be genetically determined.

Since the multiplication-depressing factor is inherited as a dominant, it is possible to compare the cellular vulnerability of the F_1 progeny (Swiss $\times$ PRI) mice with that of PRI mice, without having it complicated by the factor of viral multiplication. In repeated tests, in which approximately one million infective doses of "French neurotropic" virus were inoculated intracerebrally in groups of 20 to 30 mature PRI mice, the mortality ranged from 20 to 30 per cent, with an average of 24 per cent. Crosses made between PRI and the highly inbred CFW, Swiss mice produced 84 F_1 animals, which exhibited an average mortality of 70 per cent after intracerebral inoculation of the same large dose of "French neurotropic" virus. In litters of 7 or more mice the mortality was never less than 50 per cent, and in 2 families of 8 mice, each resulting from a cross

of PRI females with Swiss males, the mortality was 100 per cent. In general, it made no difference whether the mother was of PRI or Swiss stock. This result, which is highly significant statistically, indicates that the character of the parents influences the behavior of the progeny. When F_1 mice were backcrossed to PRI mice (not shown in figure 2), 60 per cent of the progeny (12/20) died following inoculation of the French neurotropic virus. These results strongly suggest that cellular vulnerability to the "French neurotropic" strain of yellow fever is also genetically determined. The fact that PRI parents, which were proved to be resistant, i.e., possessed a low cellular vulnerability, yielded 18 per cent of highly vulnerable progeny suggests not only that high cellular

TABLE 1

SELECTIVE ACTION OF MULTIPLICATION INHIBITION FACTOR OF PRI MICE ON VARIOUS VIRUSES PROLIFERATING IN MOUSE BRAIN

ACTION	VIRUS
Multiplication inhibited. PRI mice completely or partly resistant	Yellow Fever
	Dengue Fever
	West Nile Fever
	Japanese B Encephalitis
	St. Louis Encephalitis
	Russian Spring-Summer Encephalitis
Multiplication not affected. PRI mice fully susceptible	Western Equine Encephalitis
	Eastern Equine Encephalitis
	Venezuelan Equine Encephalitis
	Poliomyelitis
	Mouse Encephalomyelitis—"TO"
	Rabies
	Lymphocytic Choriomeningitis
	Herpes Simplex
	Vesicular Stomatitis
	Rift Valley Fever

vulnerability might involve a recessive gene or genes but also that many of the unselected PRI stock are heterozygous for this character. The F_1 progeny resulting from crossing resistant PRI mice with Swiss mice yielded 46 per cent of highly vulnerable animals, which again would suggest that the Swiss mice are probably homozygous for the recessive genes, responsible for high vulnerability, and that the resistant (low vulnerable) PRI mice are predominantly heterozygous. The progeny resulting from crossing susceptible PRI with resistant PRI contained a lower percentage (31 per cent) of highly vulnerable individuals than in the tests in which Swiss mice were used; the difference between this result and the 18 per cent of vulnerable progeny produced by resistant PRI parents is in the right direction but not large enough for statistical significance. A great deal more work will have to be done on segregating the unselected PRI mice

by individual matings with Swiss mice before further genetic analysis of the factor of cellular vulnerability will become possible. While the viral-multiplication factor yielded "all or none" results, there is an indication that cellular vulnerability is not an "all or none" character, since a small proportion of mice develop weakness or extensive paralysis of the extremities but do not die.

The genetic factor in PRI mice, which depresses multiplication of the yellow fever virus, was found to exert a similar effect on the viruses of dengue fever, West Nile fever, Japanese B, St. Louis and Russian spring-summer encephalitis, but was without effect on a large group of other viruses listed in table 1. It is of interest to note that the viruses, which are affected by this genetic factor, are also linked together by a chain of common antigens,[6] not shared by the other viruses.

TABLE 2

OCCURRENCE OF VARIANT STRAINS OF VIRUS CAPABLE OF OVERCOMING INHERITED RESISTANCE OF HOST

VIRUS	STRAIN	NO. OF PASSAGES IN SWISS MICE	BEHAVIOR IN PRI MICE — MORTALITY, %	BEHAVIOR IN PRI MICE — PEAK MULTIPLICATION IN BRAIN	PEAK MULTIPLICATION IN BRAINS OF SWISS MICE
Japanese B encephalitis	Korea	4	13	2.4, 3.0, 3.8[a]	8.0[a]
	Nakayama	43	50	3.3	7.8
	Nakayama	±70	100	7.6	9.5
St. Louis encephalitis	Winkler	8	6	3.6	7.8
	Webster No. 3	"Hundreds" over 17 years	90	4.2	9.0

[a] Reciprocal of log of LD_{50}.

Only the dengue viruses behaved like the 17 D yellow fever virus in that all the PRI mice were uniformly resistant regardless of dosage. The behavior of the West Nile, Japanese B and St. Louis viruses varied markedly with the strain of virus and the number of passages it had had in Swiss mice prior to test in PRI mice. Some of the strains killed no PRI mice, others a varying proportion, and still others killed all the animals. Table 2 presents some of the data which indicate that viruses may overcome the inherited resistance of the host in at least 2 different ways: (1) by possessing or developing variants which can kill at low levels of viral multiplication, and (2) by the development of variants which are no longer inhibited by the inherited multiplication-depressing factor. We cannot be certain that these variations in the populations of particles belonging to any one virus may not occur in nature, and, the genetically resistant mouse can be used to investigate this possibility. For example, it was found that 2 of the 3 strains of West Nile virus recovered in Dr. Paul's laboratory from Egyptian sera,[7] killed only a small proportion of PRI mice, while the

third strain (Egypt 101), in the second mouse passage, killed all PRI mice although it multiplied at a level that was only $^1/_{4000}$ of that achieved in the brains of Swiss mice.

Summary and Discussion.—These studies, in brief, have shown that an analysis of inherited resistance to viruses must take into consideration not only factors which control viral multiplication but also the phenomenon of cellular vulnerability. The available data suggest that cellular vulnerability also may be a genetically controlled character. With some strains of virus only one of these factors comes into play, with others, both. The demonstration that any one virus does not consist of a homogeneous population of particles, and that certain strains can possess or develop the capacity to overcome one or the other, or both, of these host barriers, poses a problem of considerable interest with regard to the potential periodic emergence of viruses with special epidemic or epizootic properties. These data provide a model which indicates how the viruses responsible for encephalitis and poliomyelitis may behave in populations of mixed or highly selected genetic constitution. The data also provide an explanation for the occurrence in some of the highly endemic areas of Africa of population groups which are almost uniformly resistant to yellow fever. The mixed occurrence of severe and mild infections among the South American Indians is in accord with the hypothesis that yellow fever was imported there from Africa after the discovery of America, and that not enough time has elapsed for the virus to kill off a significant number of those carrying the recessive genes for susceptibility. Finally, these studies provide us with a model of how nature prevents viruses from causing disease by allowing them to multiply just enough to produce immunity, but not enough to damage the tissues—a model that we may well investigate in great detail because an understanding of the underlying mechanisms would provide us with the ideal procedures for the chemoprophylaxis of virus diseases.

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